

RESEARCH

Open Access



Lipid-lowering properties of protein-rich mucuna product

Josiane Therese Metsagang Ngatchic^{1*}, Nicolas Yanou Njintang², Clemence Bernard³, Julius Oben⁴ and Carl Moses Mbofung¹

Abstract

Background: The objective of this study is to evaluate the effect of protein-rich mucuna product (PRMP) on lipid parameters of hyperlipidemic rats.

Methods: Hyperlipidemia was induced in male rats for 3 weeks through high-fat diet. After induction, 30 hyperlipidemic rats were divided into five groups of six rats: control group (CG) received casein and four groups received PRMP as protein source at different proportions: 8.2, 16.4, 24.6, and 32.8 % corresponding, respectively, to 25, 50, 75, and 100 % substitution of casein in the diet for 3 weeks. Lipid and oxidative stress parameters of rats were assessed.

Results: There were no significant differences in food intake and body weight loss among the experimental groups. The concentrations of the serum low-density lipoprotein cholesterol, total cholesterol, and triglycerides were lower in groups fed on PRMP 50, 75, and 100 % than in the CG group ($p < 0.05$). Histological analysis of the liver revealed that animals fed on PRMP diets presented a lower level of steatosis than the CG group. The most significant reduction of lipid parameters was obtained when PRMP was used as unique source of protein (PRMP 100 %). PRMP also influenced oxidative stress parameters as evidenced by a decrease in malondialdehyde and an increase in catalase and superoxide dismutase.

Conclusions: These findings demonstrated that PRMP exerts hypolipidemic effect; it has a metabolic effect on endogenous cholesterol metabolism and a protector effect on the development of hepatic steatosis. Our results also suggest that PRMP could manage metabolic diseases associated with oxidative stress.

Keywords: Protein-rich mucuna product, Cholesterol, Triglycerides, Steatosis, Oxidative stress

Background

Dyslipidemia is an independent and modifiable risk factor for cardiovascular diseases [1]. Its prevalence is growing in developed countries as well as in developing countries [2]. Dyslipidemia is characterized by elevated low-density lipoproteins (LDL), high triglycerides and low high-density lipoproteins (HDL) level. High levels of HDL are found consistently to be associated with long life in diverse population while LDL is the principal atherogenic component of cholesterol and major constituent of atherogenic plaques and cardiovascular diseases [3, 4]. The major patho-physiological mechanism involved in atherosclerosis is oxidative stress [5]. In this

respect, oxidatively modified LDL particles are thought to promote specifically the early development of atherosclerotic lesions [6].

Among strategies to fight against dyslipidemia and to reduce the risk of coronary disease, dietary management has shown promising results through a regulation of HDL and LDL cholesterol levels. In particular, beneficial effects on serum lipids (cholesterol-lowering effect) have been reported on our daily food seeds and seed proteins including soy bean [7], faba bean [8], amaranth [9], cow-pea [10], and pea [11].

The cholesterol-lowering effect of seeds and seed proteins has been associated with the action of components such as phytic acid, dietary fiber, saponins, phytosterols, proteins, peptides, and the amino acid profile of the proteins [12]. While cholesterol-lowering effect of many conventional legume seed protein isolates and, particularly,

* Correspondence: josianetheresa@yahoo.fr

¹Department of Food Science and Nutrition, National School of Agro-industrial Science, Ngaoundere, P.O. Box 455, Cameroon

Full list of author information is available at the end of the article



soy proteins have been widely studied, much is still to be done for under-utilized seeds of the tropical legume like-*Mucuna pruriens*. *Mucuna* seeds are very good sources of proteins (23–35 %) [13], but they contain appreciable amount of antinutrients tannins, trypsin inhibitors, polyphenols, and phytate that limit their consumption [13, 14]. In this respect, our recent studies reported that consumption of raw mucuna flour produces deleterious effects on growth, hematological parameters, and kidneys of rats [14]. In order to improve the nutritional properties of mucuna beans, we successfully produced a curd by the process of decortication, milling to produce flour, extraction of protein by thermal solubilization, and protein precipitation using citric acid. The product was not only poor in antinutrients but also contained 59–61 % proteins, and based on this [14], it was called protein-rich mucuna product (PRMP). Rats that fed PRMP exhibited blood biochemical and hematological characteristics, liver and kidney histology, and growth performance similar to casein as protein reference [14]. Moreover, compared to animals that fed on casein as protein source, PRMP induces a decrease in blood cholesterol; a property generally associated with consumption of legume seeds [8].

The present study was undertaken in an attempt to confirm the probable hypolipidemic effect of PRMP, with the objective to evaluate the effect of protein substitution in the diet by PRMP protein on the serum's lipidemic profile of hyperlipidemic rats.

Methods

Production of PRMP and amino acid profile determination

Mucuna pruriens var. *pruriens* seeds were harvested from an experimental farm located at the University of Ngaoundere (Cameroon). Seeds of *Mucuna* were used to produce PRMP using the hydrothermal solubilization and acid precipitation of proteins as recently described by Ngatchic et al. [14]. The amino acid composition of PRMP was determined by HPLC using RP-18.220 column (PTC RP-18.220 mm, 2, 1 mm I.D, Applied Biosystem, Appleton Bio Corps, Foster City, USA) after hydrolyzing the samples with 6 N HCl at 105 °C for 24 h.

In vitro antioxidant activity

Preparation of extract

0.5 g of PRMP powder was extracted twice with 25-mL ethanol (70 %) at 25 °C for 24 h. Extract was filtered with Whatman N 1 filter paper, and the filtrate was stored at –20 °C for determination of antioxidant activity.

Determination of DPPH radical scavenging activity of PRMP

The free-radical scavenging activity of the PRMP was measured in vitro by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay according to the method described by Hatano et al. [15]. 0.25 mL of DPPH (0.1 mM in

methanol) was mixed with 0.25 mL of PRMP extract. The reaction mixture was shaken well and incubated in the dark for 60 min at room temperature. Then, the absorbance was taken at 517 nm. Ethanol (0.25 mL) was used as control. The antioxidant activity was given as the inhibition rate of DPPH radicals. The results were expressed in milligram trolox per 100 g of PRMP (dry weight).

$$\text{Inhibition rate (\%)} = \frac{\text{Control absorbance} - \text{Extract absorbance}}{\text{Control absorbance}}$$

Ferric iron-reducing activity

The ability of PRMP extract to reduce iron(III) to iron(II) was evaluated following the method of Oyaizu [16]. An aliquot of 1 mL of PRMP extract was dissolved in distilled water and mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of aqueous $\text{K}_3\text{Fe}(\text{CN})_6$ (1 %) solution and incubated for 30 min at 50 °C. 2.5 mL of trichloroacetic acid 10 % were added, and the mixture centrifuged for 10 min at 2000g. 2.5 mL aliquot of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of aqueous FeCl_3 0.1 %. The absorbance was taken at 700 nm. Ferric iron-reducing activity was determined as ascorbic acid equivalents (mg ascorbic acid/100 g of PRMP).

Diet formulation and animal experiment

Forty-two male Wistar rats weighting 225–230 g obtained from the Animal House of National School of Agro-Industrial Sciences were used for the experiment. The animals were housed individually in metabolic cages maintained at a temperature of 23 ± 3 °C with alternate periods of 12-h light and dark with free access to water and food. The animals were randomly grouped into seven groups of six animals each. After an acclimatization period of 4 days during which the rats were fed on standard diet with casein as protein source, to induce hyperlipidemia, six groups of rats were fed ad libitum with a high-fat diet rich in saturated fatty acids and cholesterol during the same period the remaining group was fed with standard diet formulated following Macarulla et al. [8], with modifications reported in Table 1. At the end of this period, one group of rats fed with high-fat diet and the group fed with standard diet were killed to check whether hyperlipidemia had been achieved.

The livers of these rats were removed and submitted to histological evaluation to check steatosis. After confirmation of steatosis and hyperlipidemia, the so-called hyperlipidemic rats (five groups) were submitted to normal food regimes ad libitum for 3 weeks with proteins substituted with PRMP at 0, 25, 50, 75, and 100 %. The 0 % substitution group received 100 % casein as protein source and was considered as control group (CG), while

Table 1 Formulation of diets for induction of hyperlipidemia

Ingredients	Standard diet	High-fat diet
Casein	200	200
Soy bean oil	50	50
Coconut oil	–	250
Cholesterol	–	10
Vitamin mix	10	10
Mineral mix	50	50
L-methionine	1	1
Sucrose	50	50
Cellulose	50	–
Corn starch	589	379
Energy (MJ/kg)	18	25

the 100 % substitution received essentially PRMP as protein source. The diets were formulated to provide the same amount of proteins as shown in Table 2. In this respect for 100 g food formulated, CG received 20 g casein as protein source, the four remaining groups received the amount of PRMP of 8.2, 16.4, 24.6, and 32.8 g corresponding, respectively, to 25, 50, 75, and 100 % of substitution. Food intake, individual rat body weight, and feed waste were measured and recorded per 2 days and used in calculating day's weight gain or loss.

Blood sample collection, serum lipid profile, and histopathological analysis

At the end of the 3-week experiment, rats were fasted for 14 h, the blood samples were collected, and the liver and kidney were removed. Serum lipid profile was done as previously reported by Ngatchic et al. [14]. In this respect, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) in serum were

Table 2 Formulation of experimental diets

Ingredients	CG	Protein-rich mucuna products (%)			
		25 %	50 %	75 %	100 %
Casein	200	150	100	50	–
PRMP	–	82	164	246	328
Soy bean oil	50	50	50	50	50
Vitamin mix	10	10	10	10	10
Mineral mix	50	50	50	50	50
L-methionine	1	1	1	1	1
Sucrose	50	50	50	50	50
Cellulose	50	–	–	–	–
Corn starch	589	608	575	543	511
Energy (MJ/kg)	17.8	17.9	18.6	18.9	17.98
Arginine/lysine	0.46	0.68	0.94	1.19	1.36

CG control group, *n* = 6

determined as described earlier [14]. In order to observe the steatosis, the histological examination of liver was performed; in the procedure, organs were fixed in formaldehyde (10 %) and the tissues subsequently dehydrated in upgraded concentrations of ethanol (10–90 %), cleaned in xylene, impregnated, and embedded in paraffin. Sections of 5 µm were cut using a microtome, stained with hematoxylin and eosin stains. Light microscopic (microscope OPTIKA DM-20, SN 223932 Italy; camera CM-D OLYMPUS) examination of multiple tissue sections from the liver in all animal groups was performed, and image representatives of the typical histological profile were examined.

Tissue preparation and determination of in vivo antioxidant activity

Liver and kidney removed were rinsed with NaCl (0.9 %) solution. Tissues were minced and homogenized (10 %w/v) in ice-cold potassium phosphate buffer (0.1 M, pH 7.4). The homogenate was centrifuged at 3000g for 10 min at 4 °C; the resultant supernatant was used for the determination of antioxidant activity.

Measurement of lipid peroxidation

Lipid peroxidation was evaluated with the Yagi [17] method. This method depends on the formation of malondialdehyde (MDA) as an end product of lipid peroxidation. The amount of malondialdehyde was determined by the spectrophotometric method at 532 nm after the reaction with thiobarbituric acid. The level of malondialdehyde was determined using the molar extinction coefficient of chromophore ($1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$).

Catalase activity

Catalase activity was assayed by the method of Sinha [18] which is based on the formation of chromic acetate from dichromate and glacial acetic acid in the presence of hydrogen peroxide. Chromic acetate produced was measured using spectrophotometer at 620 nm. One enzyme unit was defined as the amount of enzyme which catalyzed the oxidation of 1 µmol H₂O₂ per min under assay conditions. The activity was expressed in terms of units per milligram of protein.

SOD

The superoxide dismutase (SOD) activity was determined by the spectrophotometric method based on the inhibition of adrenaline oxidation to adrenochrome [19]. Briefly, 0.2 mL of sample was diluted in 3 mL of carbonate buffer, pH 0.2, and placed into quartz spectrophotometer cuvette. The reaction was started by adding 0.3 mL of adrenalin 0.3 mM solution in 10 mM HCl. Adrenaline oxidation leads to the formation of the colored product, adrenochrome, which is detected by the

spectrophotometer. In the basic pH, the adrenalin was spontaneously oxidized, with the kinetics recorded by measuring the increase of absorbance at 480 nm over time. The kinetics of adrenalin oxidation in the presence of the sample was compared with the oxidation rate of adrenalin alone. A unit of SOD is defined as the amount of enzyme that inhibits the rate of adrenaline oxidation by 50 %. The result was expressed in microunit per milligram of protein. Protein was determined using the method of Lowry et al. [20].

Statistical and data analyses

The statistical analyses were performed using the Statgraphics software, version 5.0. The values were presented as means with their standard deviation (\pm SD). One-way analysis of variance (ANOVA) was performed to test the significance of differences ($p < 0.05$) between groups. When the difference was significant, a Duncan multiple comparison range test was used as a post hoc test. Correlation was performed between variables to evaluate their relationship (significant correlation at $p < 0.001$), while a principal component analysis was used for visualization of difference between treatments.

Results

Amino acid profile of PRMP

The amino acid composition of PRMP and the FAO/WHO [21] reference are shown in Table 3. It can be seen that compared to FAO/WHO(2007) reference, PRMP was

Table 3 Amino acid composition of protein-rich mucuna product (PRMP) (mg/g protein)

Amino acids	PRMP	FAO/WHO pattern (2007)
Isoleucine	78.9	30
Leucine	85.7	59
Lysine	42.9	45
Histidine	21.0	15
Valine	42.8	39
Threonine	35.1	23
Phenylalanine + tyrosine	51.79	38
Methionine + cystine	3.4	22
Tryptophan	nd	6
Alanine	25.9	–
Proline	20.6	–
Arginine	58.4	–
Serine	40.8	–
Glycine	57.7	–
Aspartic acid	191.3	–
Glutamic acid	170.4	–

nd not determined

rich in most essential amino acids (isoleucine, leucine, histidine, valine, threonine, phenylalanine, and tyrosine) with the exception of sulfur amino acids (cysteine and methionine) which were very low. The level of lysine was equally approaching that of the reference. Of the amino acids analyzed, aspartic acid and glutamic acid were the most abundant amino acids. These observations are common not only with legume seeds protein such as chickpea [22], cowpea, and lupin [23] but also with most plant proteins as tubers [24]. The relatively high levels of amino acids offer to plant proteins an acidic character which is characterized by an isoelectric point around pH 4 and 5. In this respect, solubility is mostly high at neutral pH which enables an aqueous extraction of proteins with good yield. This is important if protein has to be used for human nutrition, the extraction conditions need to avoid additives and respect physiologic conditions.

In vitro antioxidant activity

Results of this study reveal that antioxidant activity of PRMP is 6.15 ± 0.03 mg trolox equivalent/100 g of dried matter for DPPH activity and 3.68 ± 0.06 mg ascorbic acid equivalent/100 g of dried matter for ferric iron-reducing activity, showing that the antioxidant potential of PRMP is not negligible. This must be considered since it already has a capacity to scavenge free radical and reducing metal.

Effect of high-fat diet on lipid profile, body and liver weight, and antioxidant enzymes

The lipid profile of rats fed with high-fat diet is presented in Table 4, as well as the food intake and weight gain. As compared to rats submitted to standard diet (Table 4), the total cholesterol, LDL cholesterol, and triglyceride levels were two- to fourfold higher ($p < 0.005$) in rats fed with high-fat diet while HDL cholesterol was significantly lower, thus confirming that hyperlipidemia was effectively induced. This is likely subsequent to the high level of fat in the regime as no significant difference was observed on the food intake between both food regimes. Generally, rats fed with high-fat diet increased weight of about 59 %. The present study also showed

Table 4 Food intake, weight gain, and serum lipid levels of rats fed on the standard and high-fat diet for 3 weeks

Parameters	Standard diet	High-fat diet
Food intake (g/day)	$18.9 \pm 5.04a$	$18.9 \pm 1.09a$
Weight gain (g)	$22.2 \pm 0.57a$	$35.6 \pm 0.65b$
Total cholesterol (mg/dL)	$63.2 \pm 2.76a$	$182.1 \pm 4.13b$
HDL cholesterol (mg/dL)	$26.6 \pm 1.80a$	$17.0 \pm 0.76b$
LDL cholesterol (mg/dL)	$29.6 \pm 1.47a$	$124.5 \pm 3.53b$
Triglycerides (mg/dL)	$95.2 \pm 1.37a$	$176.7 \pm 3.87b$

Mean \pm SD followed by different letters (a–b) in the same line are significantly different ($p < 0.05$); $n = 6$

significant decrease of catalase and superoxydase activities in serum, liver, and kidneys of animals fed with high-fat diet ($p < 0.005$) than group fed with standard diet (Table 5). Significant increase of tissues and serum level of malondialdehyde was also observed in rats fed with high-fat diet. These results assure that rats fed with high-fat diet are stressed and have dyslipidemia.

Effect of PRMP on food intake, body, and liver to body weights of hyperlipidemic rats

During this experiment, a loss in rat weight (mean weight loss equal to 4.9 %) was observed irrespective of the protein sources (Table 6). In addition, the mean food intake was 18.9 g/day/rat, and no significant difference was observed on food intake among groups. However, as the level of PRMP increased in the diet, the liver to body weight ratio diminished progressively. The diminution (4.6 %) was not significant up to 75 % substitution, while at 100 % substitution, up to 11 % significant reduction was observed. The reduction in the ratio probably reflected the reduction in liver fat subsequent to consumption of PRMP. This was confirmed by the histopathology of the liver (Fig. 1) which showed a progressive reduction of fat deposit in hepatocytes as the level of PRMP increased in the diet. At 100 % substitution where PRMP was the unique source of protein, a clear reduction in fat deposition area in the liver can be observed in conformity with the reduction in rat weight observed above.

Effect of PRMP on serum lipid levels

Figure 2 shows the effect of PRMP as protein source on the total cholesterol, LDL, HDL cholesterol, and triglycerides of hyperlipidemic rats. While no significant change was observed on the HDL cholesterol, it was observed that the total cholesterol, LDL cholesterol, and triglyceride progressively diminished as the percentage of PRMP increased. In this respect, at 0 and 25 % PRMP, no significant difference in these parameters was observed. But at 50 % substitution, a significant drop in total and LDL cholesterol and

Table 6 Food intake, body weight loss, and liver weight/body weight ratio of rats fed on standard diet and protein-rich mucuna product

Groups	Food intake (g/d)	Body weight loss (g)	Liver weight/body weight
CG	18.88 ± 0.83a	4.77 ± 0.44a	3.66 ± 0.06a
PRMP 25 %	18.50 ± 0.93a	4.97 ± 0.47a	3.62 ± 0.01a
PRMP 50 %	18.77 ± 1.05a	5.01 ± 0.65a	3.53 ± 0.08a
PRMP 75 %	18.87 ± 1.25a	5.21 ± 1.03a	3.50 ± 0.05a
PRMP 100 %	18.64 ± 1.4a	4.85 ± 0.98a	3.28 ± 0.04b

Values are expressed as mean ± SD, $n = 6$ in each group. Means followed by different letters (a–b) in the same column are significantly different ($p < 0.05$)

triglycerides was observed, and this was continued as the substitution increased to 75 and 100 %.

Figure 3 presents principal component analysis of serum lipid parameters. The principal component analysis was performed on data to visualize the effect of substituting casein in the diets by PRMP protein on the physiological and blood lipid parameters of rats. The correlation circle revealed that triglyceride, LDL, and total cholesterol were close together, consequence of positive correlation between them. In this respect, the variable triglycerides were linearly correlated to LDL cholesterol ($r = 0.95$; $p < 0.001$), total cholesterol ($r = 0.97$; $p < 0.001$), and weight to liver ratio ($r = 0.90$; $p < 0.001$). In addition, the level of PRMP in the diets was negatively correlated with triglycerides ($r = 0.85$; $p < 0.001$), total cholesterol ($r = 0.87$; $p < 0.001$), LDL cholesterol ($r = 0.92$; $p < 0.001$), and liver ratio ($r = 0.88$; $p < 0.001$). These parameters (total cholesterol, LDL cholesterol, triglyceride, and liver to body weight ratio) were associated with the principal component (PC) 1 (66.3 % variation) contributing for 21 % each. HDL cholesterol also had a contribution (16 %) with the PC1 axis, which however was opposed to the other lipid profile parameters. The PC2 contributed for 20.3 % variation and was related mostly to weight loss (60 %), food intake (20 %), and to some extent HDL (14 %). The biplot in Fig. 3 showed that the rat groups fed with varying levels of PRMP in their diets differed mainly according to the PC1 axis, as a consequence of the variation in their lipid profile. It can then be seen from this plot that as the proportion of PRMP increased in the diet from 0 to 75 %, the rat groups move from right to upper-left of the PC1 axis, which means from high level of triglycerides, LDL, and total cholesterol toward high level of HDL, high weight loss, and food intake. At 100 % PRMP, the LDL, HDL, and total cholesterol continuously decreased, while a drop in the weight loss is observed and consequently, the rat group 100 % is at the under-left of the PC1 axis. Even though the PC2 axis has less contribution to the variance among the rat

Table 5 Effect of high-fat diet on stress parameters of rats

	Parameters	Standard diet	High-fat diet
Serum	MDA (μM)	1.78 ± 0.02a	2.62 ± 0.05b
	CAT (Unit/mg)	554.42 ± 3.29a	543.06 ± 2.10b
	SOD ($\mu\text{Unit/mg}$)	104.08 ± 1.99a	81.82 ± 2.58b
Liver	MDA (μM)	2.11 ± 3.35a	3.11 ± 1.88b
	CAT (Unit/mg)	659.22 ± 1.94a	649.09 ± 1.17b
	SOD ($\mu\text{Unit/mg}$)	114.50 ± 4.38a	93.74 ± 4.51b
Kidney	MDA (μM)	2.06 ± 0.09a	2.83 ± 0.03b
	CAT (Unit/mg)	577.67 ± 3.29a	567.55 ± 1.04b
	SOD ($\mu\text{Unit/mg}$)	101.42 ± 2.02a	96.64 ± 1.45b

Values are expressed as mean ± SD, $n = 6$ in each group. Means followed by different letters (a–b) in the same row are significantly different ($p < 0.05$)
MDA malondialdehyde, CAT catalase, SOD superoxide dismutase

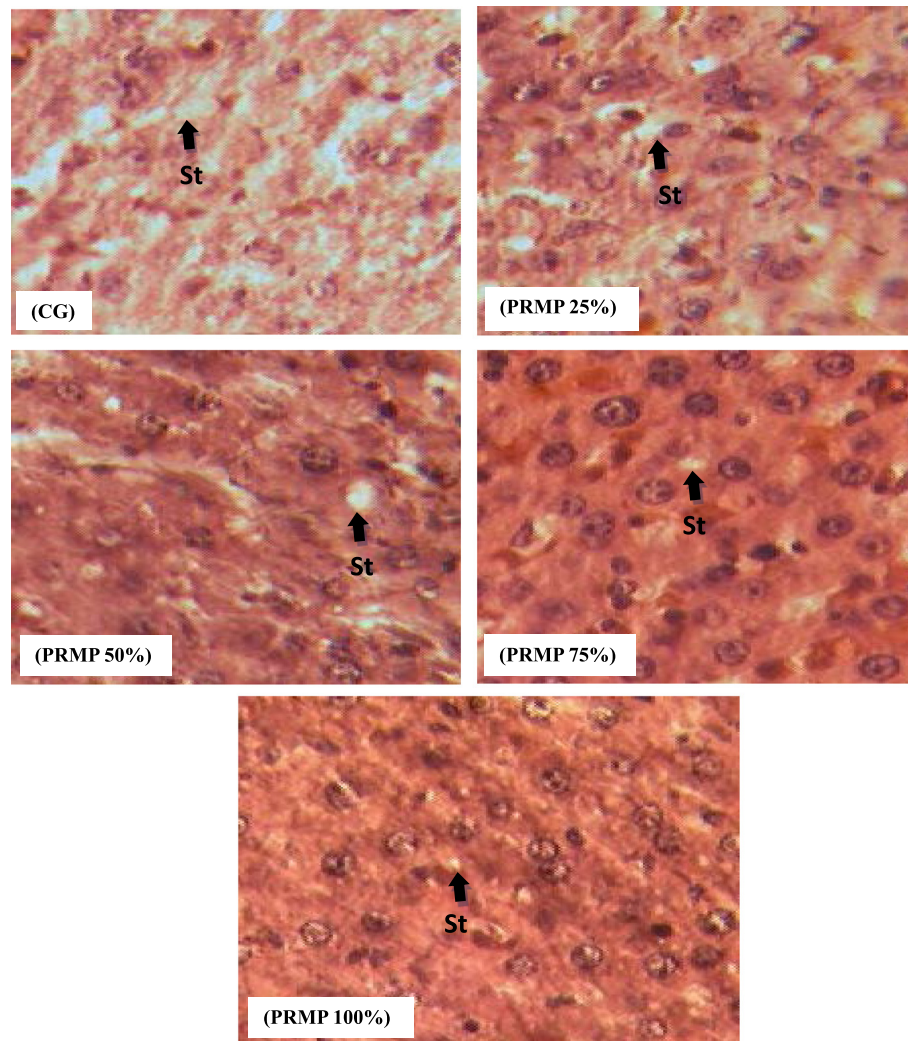


Fig. 1 Histological section of the liver illustrating lipid accumulation in rats fed with PRMP at different proportions (25, 50, 75, and 100 %). *St* steatosis (magnification $\times 400$)

groups, the change in direction from 75 to 100 % needs to be investigated. One question to be address here is the eventual toxicity associated with consumption of PRMP as source of proteins and the related change in weight.

Effect of PRMP on antioxidant enzymes

The antioxidant content in rat organs as affected by level of PRMP is shown in Table 7. The change in proportion of PRMP up to 75 % had no significant effect on the antioxidant enzymes superoxide dismutase and catalase in serum, liver, and kidney. At 100 % substitution with PRMP, a significant increase ($p < 0.005$) in these antioxidant enzymes was observed. Concerning MDA, a significant decrease was observed at substitutions 75 and 100 % in all the organs studied.

Discussion

Hyperlipidemia is a major contributor to the pathogenesis of cardiovascular diseases which is a leading health problem in the world. In this respect, strategies to improve the blood lipid's profile were associated with the reduction of these diseases [25]. The main finding of the present study was that PRMP decreased the blood concentration of total cholesterol, LDL cholesterol, triglycerides, and the fat liver accumulation. More precisely, the total cholesterol of the rat groups fed diets with PRMP at 25, 50, 75, and 100 % substitution of casein as protein source diminished for 0.01, 3.6, 11, and 30.7 %, respectively. Similarly, the LDL cholesterol level decreased for 0.5, 4, 21, and 37.8 %, respectively, as the level of PRMP increased in the diet. Similar reduction in total and LDL cholesterol has been reported for other plant protein isolates as cowpea [7], amaranth [26], and pea [27]. Several

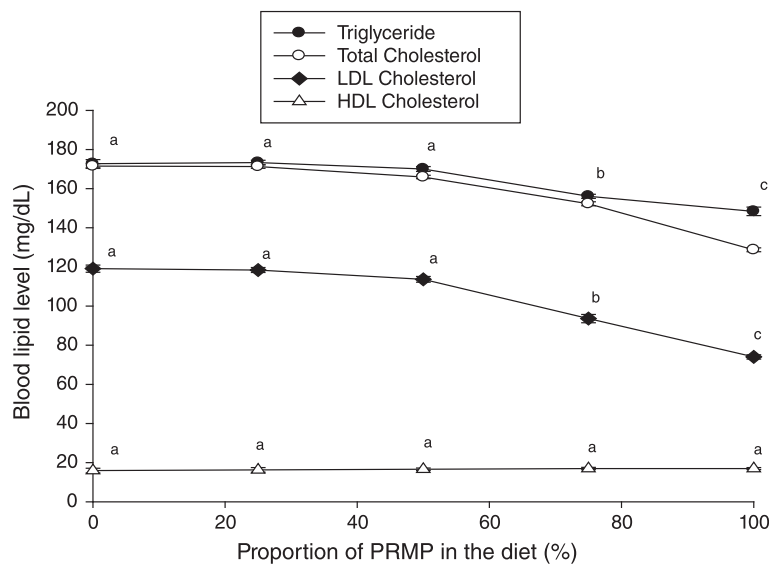


Fig. 2 Blood lipid profile in hyperlipidemic rats fed with standard and PRMP diets. Mean \pm standard deviation for each lipid parameter affected by different letters (a–d) are significantly different ($p < 0.05$); $n = 6$

mechanisms of action have been suggested to explain the reduction of cholesterol and triglycerides in rat fed with protein isolates: the amino acid profile, the presence of bioactive peptides, or other molecules.

Among the mechanisms of hypolipidemic action of legume seed protein isolates, the composition in amino acids has been widely reported. In this respect, Carroll

and Kurowska [7] and Dabai et al. [28] reported that plants with low methionine concentrations and elevated arginine to lysine ratio exhibited hypolipidemic properties. The results obtained in the present work apparently follow this trend. In fact, the analysis of the amino acid profile of the PRMP proteins revealed that the arginine to lysine ratio was 1.36, higher than the value 0.46

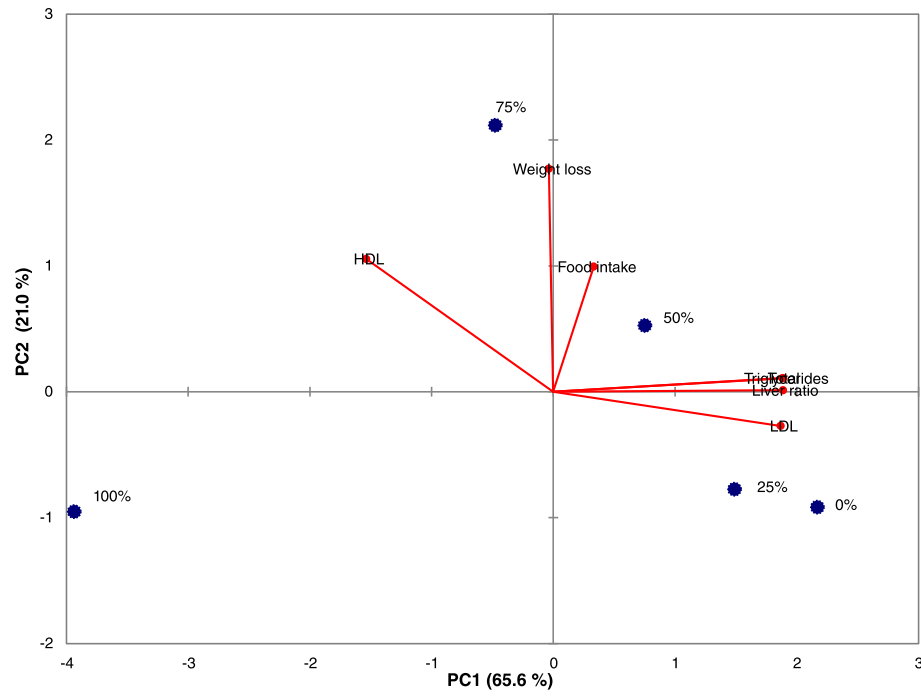


Fig. 3 Biplot of dependant variables and rat groups (shown in blue color) on varimax-rotated principal component axes

Table 7 Effect of proportion of PRMP as protein source in their diet on the oxidative stress parameters of experimental rats

Proportion of PRMP (%)	Catalase (Unit/mg)			Superoxide dismutase (μ Unit/mg)			Malondialdehyde (μ M)		
	Serum	Liver	Kidney	Serum	Liver	Kidney	Serum	Liver	Kidney
0	548.68 \pm 3.48a	654.15 \pm 5.01a	572.33 \pm 5.02a	91.78 \pm 1.06a	106.67 \pm 1.67a	99.05 \pm 0.70a	2.14 \pm 0.01a	2.76 \pm 0.01a	2.69 \pm 0.01a
25	550.51 \pm 5.07a	654.06 \pm 6.92a	572.59 \pm 6.58a	91.69 \pm 1.00a	107.07 \pm 1.11a	99.37 \pm 0.57a	2.13 \pm 0.02a	2.74 \pm 0.03a	2.69 \pm 0.03a
50	551.75 \pm 5.10a	654.50 \pm 6.56a	572.39 \pm 6.73a	91.42 \pm 1.85a	107.31 \pm 0.57a	99.08 \pm 0.58a	2.13 \pm 0.03a	2.73 \pm 0.02a	2.69 \pm 0.02a
75	548.21 \pm 5.02a	655.85 \pm 4.08a	572.29 \pm 6.76a	92.81 \pm 1.34a	107.39 \pm 0.63a	100.07 \pm 1.40a	2.02 \pm 0.02b	2.65 \pm 0.03b	2.60 \pm 0.02b
100	565.14 \pm 3.83b	670.32 \pm 5.40b	592.62 \pm 6.49b	96.89 \pm 1.75b	112.30 \pm 1.66b	110.83 \pm 1.81b	1.94 \pm 0.01c	2.5 \pm 0.01c	2.53 \pm 0.01c

Values are expressed as mean \pm SD, $n = 6$ in each group. Means followed by different letters (a–c) in the same column are significantly different ($p < 0.05$)

reported for casein. Some other researchers associated with the hypocholesterolemic effect of legume seed protein isolates to the presence of bioactive peptides. Cho et al. [29] and Rigamonti et al. [27] reported incomplete digestion of legume seed proteins in the digestive tract, and liberation of specific peptides which reduce the levels of cholesterol, probably by regulation of the activity of HMG-CoA reductase, a key enzyme in the synthesis of cholesterol, and the activity of LDL receptor. It has been demonstrated that α and α' subunits of 7S soy globulin may reduce blood cholesterol [30] while the peptide conglutin of protein lupin has been reported to increase LDL receptor activity [31]. The analysis of residual peptides upon digestion of PRMP in the digestive tract and its cholesterol-lowering action has not been investigated yet. Several studies revealed that proteins do not exhibit hypolipidemic activities but rather secondary metabolites (such as phytates and polyphenols) that accompany them. Convincing experimental evidence has been presented for depressing increase in hepatic lipids and in the hepatic activities of lipogenic enzymes (HMG-CoA reductase, fatty acid synthetase) by dietary phytate in animal [32–34]. In addition, polyphenols induce metabolic hypolipidemic effect mainly by their ability to reduce cholesterol acyltransferase and HMG-CoA reductase activities. In relation to these molecules, Rigamonti et al. [27] reported hypotriglyceridemic effect of pea protein isolates through mechanism of inactivation of enzymes involved in fatty acid synthesis such as fatty acid synthetase and stearoyl-CoA desaturase. Our recent investigations [14] revealed that PRMP contained residual level of phytate (2.56 %) and polyphenols (1.04 %) which could be responsible of the hypolipidemic activity of PRMP. Based on the above, the hypolipidemic action of PRMP could result from a synergistic bioactive phytochemical phytate, polyphenols, and amino acids leading to a reduction of fat accumulation in the liver and body weight.

Accumulation of fat in the liver (steatosis) is a major characteristic of dyslipidemia and metabolic syndrome; thus, the reduction of steatosis is a good indicator of the management of dyslipidemia. The difference in hepatic lipid accumulation between the rats fed is varying.

PRMP regime clearly highlighted the reduction of hepatic fat with increased level of PRMP in the diet (Fig. 1). Compared to the livers of the rats fed with 100 % casein as protein source that showed widespread fat globules of broad size, steatosis size was reduced with increasing proportion of PRMP in the diet. There was a large reduction of fat deposit areas in the liver of the animals fed with PRMP 100 %, thus justifying the decrease in liver weight. Such regulation has been demonstrated in rat hepatocytes and adipocytes following ingestion of soy proteins through modulation of the secretion of several adipokines and free fatty acids (FFA) [35, 36]. A sterol regulatory element-binding protein (SREBP)-1-dependent mechanism has been reported for the regulation of fatty acid synthesis upon ingestion of protein isolates [37].

A number of oxygenated compounds are produced during the attack of free radicals against membrane lipoproteins, proteins, and polyunsaturated fatty acids. One of them is malondialdehyde which can be used as an indicator of oxidative stress, as its concentration in plasma increases as the result of free-radical processes. The antioxidant system enables transformation of reactive oxygen species into inactive and harmless compounds. Antioxidant enzymes: superoxide dismutase, glutathione peroxidase, and catalase provide primary defense against reactive oxygen species. Superoxide dismutase can selectively scavenge a superoxide radical by catalyzing its dismutation to hydrogen peroxide and molecular oxygen, while glutathione peroxidase and catalase serve to decompose hydrogen peroxide to the unreactive species [38]. Since antioxidant enzymes are primary defense against reactive oxygen species, increase of enzymes activities (catalase and superoxide dismutase) and lower levels of the malondialdehyde observed on rats fed with PRMP show that consumption of PRMP could prevent oxidation of organism compounds like low-density lipoprotein and thus prevent many diseases such as cardiovascular diseases.

Conclusions

The results of the present study indicate that consumption of diet rich in fat results in triglyceride deposition

in the liver, increase in blood total cholesterol, LDL cholesterol, and triglycerides. Increase in proportion of protein-rich mucuna product (PRMP) as protein source in diet results in a linear decrease of LDL and total cholesterol, triglycerides, and liver steatosis. However, the mechanism of cholesterol-lowering properties needs to be investigated. In addition, since oxidative stress is generally associated with metabolism disorder, improving antioxidant activities and reduced levels of malondialdehyde conclude that PRMP could play an important role in management of metabolic diseases, like cardiovascular diseases.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JTMN carried out the experimental work, data collection and evaluation, literature search, and draft preparation. NYN performed the statistical analysis and contributed to the draft manuscript. CB and JO were responsible for the critical review, intellectual input in discussion, and overall presentation of paper. CMM supervised the work and refined the manuscript for publication. All authors approved the final manuscript.

Authors' information

JTMN is a lecturer at the Department of Food Science and Nutrition, National School of Agro-industrial Science.

NYN is an associate professor at the Department of Biological Sciences, Faculty of Sciences.

CB is a researcher at the Industrial School of Biology, EBNnov Laboratory.

JO is a professor at the Department of Biochemistry, Faculty of Sciences.

CMM is a professor at the Department of Food Science and Nutrition, National School of Agro-industrial Science.

Acknowledgements

The authors gratefully acknowledge the technical assistance of the laboratory staff from the Department of Food Science and Nutrition of National School of Agro-industrial Sciences of Ngaoundere University.

Author details

¹Department of Food Science and Nutrition, National School of Agro-industrial Science, Ngaoundere, P.O. Box 455, Cameroon. ²Department of Biological Sciences, Faculty of Sciences, University of Ngaoundere, Ngaoundere, P.O. Box 454, Cameroon. ³Industrial school of Biology, EBNnov Laboratory, 32 boulevard du port, 95094 Cergy, Pontoise Cedex, France. ⁴Department of Biochemistry, Faculty of Sciences, University of Yaounde, Yaounde, P.O. Box 812, Cameroon.

Received: 10 October 2015 Accepted: 14 April 2016

Published online: 25 May 2016

References

- Mandukhail Saf-ur R, Nauman A, Anwarul-Hassan G. Studies on antidyslipidemic effects of *Morinda citrifolia* (noni) fruit, leaves and root extracts. *Lipids Health Dis*. 2010;9:88–94.
- World Health Organization. Raised cholesterol. Global health observatory-risk factors. 2014.
- Schwingshackl L, Hoffmann G. Comparison of the long-term effects of high-fat low-fat diet consumption on cardiometabolic risk factors in subjects with abnormal glucose metabolism: a systematic review and meta-analysis. *Br J Nutr*. 2014;111:2047–58.
- Ballantyne CM. Treatment of dyslipidemia to reduce cardiovascular risk in patients with multiple risk factors. *Clinical Cornerstone*. 2007;8:606–13.
- Anjaneyulu M, Chopra K. Quercetin, antioxidant bioflavonoids, attenuates diabetic nephropathy in rats. *Clin Exp Pharmacol Phys*. 2004;31:244–8.
- Ijeh I, Ejike CEC. Current perspectives on the medicine potentials of *V. amygdalina* Del. *J Med Plant Res*. 2011;5:1051–61.
- Carroll KK, Kurowska EM. Soy consumption and cholesterol reduction: review of animal and human studies. *J Nutr*. 1995;125:594–7.
- Macarulla MT, Medina C, DeDiego MA, Chavarri M, Zulet MA, Martinez JA, Noel Suberville C, Higuere P, Portillo MP. Effects of whole seed and protein isolate of faba bean (*Vicia faba*) on the cholesterol metabolism of hypercholesterolemic rats. *Br J Nutr*. 2001;85:607–14.
- Plate AYA, Arêas JAG. Cholesterol-lowering effect of extruded amaranth (*Amaranthus caudatus* L.) in hypercholesterolemic rabbits. *Food Chem*. 2002;76:1–6.
- Frota KMG, Mendonça SA, Saldiva PHN, Cruz RJ, Areas JAG. Cholesterol-lowering properties of whole cowpea seed and its protein isolate in hamsters. *J Food Sci*. 2008;73:235–40.
- Spielmann J, Stangl G, Eder K. Dietary pea protein stimulates bile acid excretion and lowers hepatic cholesterol concentration in rats. *J An Phys An Nutrition*. 2008;92:683–93.
- Reynolds K, Chin A, Lees KA, Nguyen A, Bujnowski D, He J. A meta-analysis of the effect of soy protein supplementation on serum lipids. *Am J Card*. 2006;98:633–40.
- Mugendi JB, Njagi EM, Kuria EN, Mwasaru MA, Mureithi JG, Apostolides Z. Effects of processing on protein quality and anti-nutrients content of mucuna bean (*Mucuna pruriens* L.). *Afr J Food Sci*. 2010;4:156–66.
- Ngatchic JTM, Sokeng SD, Njintang NY, Maoundombaye T, Oben J, Mbofung CMF. Evaluation of some selected blood parameters and histopathology of liver and kidney of rats fed protein-substituted mucuna flour and derived protein rich product. *Food Chem Toxicol*. 2013;57:46–53.
- Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y, Yasuhara T, Yoshida T, Okuda T. Effects of the interaction of tannins with co-existing substances VI. Effects of tannins and related polyphenols on superoxide anion radical, and on 1,1-diphenyl-2-picrylhydrazyl radical. *Chem Pharm Bull*. 1989;37:2016–21.
- Oyaizu M. Studies on products of browning reaction: antioxidative activity of products of browning reaction. *Japanese J Nutr*. 1986;40:307–15.
- Yagi K. A simple fluorimetric assay for lipoperoxide in blood plasma. *Biochem Med*. 1976;10:339–52.
- Sinha KA. Colorimetric assay of catalase. *Anal Biochem*. 1972;47:387–94.
- Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal Biochem*. 1971;44:276–87.
- Lowry OH, Rosebrough NJ, Farr AL, Randall JL. Protein measurement with Folin phenol reagent. *J Biol Chem*. 1951;193:265–75.
- FAO/WHO. Protein and amino acid requirements in human nutrition. Report of a joint WHO/FAO/UNU Expert Technical Report. 2007; Series 935. *Chole-Doc* N 111.
- Sanchez-Vioque RA, Clemente J, Vioque J, Millan F. Protein isolates from chickpea (*Cicerarietinum* L.): chemical composition, functional properties and protein characterization. *Food Chem*. 1999;64:237–43.
- El-Adawy TA, Rahma EH, El-Bedawey AA, Gafar AF. Nutritional potential and functional properties of sweet and bitter lupin seed protein isolates. *Food Chem*. 2001;74:455–62.
- Njintang YN, Boudjeko T, Tatsadjieu NL, Nguema-Ona E, Scher J, Mbofung CMF. Compositional, spectroscopic and rheological analyses of mucilage isolated from taro (*Colocasia esculenta* L. Schott) corms. *J Food Sci Technol*. 2014;51:900–7.
- Bertolotti M, Maurantonio M, Gabbi C, Anzivino C, Carulli N. Hyperlipidemia and cardiovascular risk. *Pharm Therapy*. 2005;22:23–30.
- Mendonça S, Paulo HS, Robison J, Cruz JAGA. Amaranth protein presents cholesterol-lowering effect. *Food Chem*. 2009;116:738–42.
- Rigamonti E, Parolini C, Marchesi M, Diani E, Brambilla S, Sirtori CR, Chiesa G. Hypolipidemic effect of dietary pea proteins: impact on genes regulating hepatic lipid metabolism. *Mol Nutr Food Res*. 2010;54:24–30.
- Dabai FD, Walker AF, Sambrook IE, Welch VA, Owen RW, Abeyasekera S. Comparative effects on blood lipids and faecal steroids of five legume species incorporated into a semipurified hypercholesterolaemic rat diet. *J Agric Food Chem*. 1996;75:557–71.
- Cho SJ, Juillerat MA, Lee CH. Identification of LDL-receptor transcription stimulating peptides from soybean hydrolysate in human hepatocytes. *J Agric Food Chem*. 2008;56:4372–6.
- Lovati MR, Manzoni C, Gianazza E, Arnoldi A, Kurowska E, Carroll KK, Sirtori CR. Soy protein peptides regulate cholesterol homeostasis in Hep G2 cells. *J Nutr*. 2000;130:2543–9.
- Sirtori CR, Lovati MR, Manzoni C, Castiglioni S, Duranti M, Magni C, Morandi S, D'Agostina, Arnoldi A. Proteins of white lupin seed, a naturally isoflavone poor legume, reduce cholesterolemia in rats and increase LDL receptor activity in HepG2 cells. *J Nutr*. 2004;134:18–23.
- Katayama T. Effects of dietary myo-inositol or phytic acid on hepatic concentrations of lipids and hepatic activities of lipogenic enzymes in rats fed on corn starch or sucrose. *Nutr Res*. 1997;17:721–8.

33. Sung-Hyen L, Hong-Ju P, Hye-Kyung C, So-Young C, Hyun-Jin J, Soo-Muk C, Dae-Yong K, Min-Soo K, Hyun SLj. Effects of dietary phytic acid on serum and hepatic lipid levels in diabetic KK mice. *Nutr Res.* 2005;25:869–76.
34. Sung-Hyen L, Hong-Ju P, Hye-Kyung C, So-Young C, Hyun-Jin J, Soo-Muk C, Dae Yong K, Min-Soo K, Hyun SLj. Dietary phytic acid improves serum and hepatic lipid levels in aged ICR mice fed a high-cholesterol diet. *Nutr Res.* 2007;27:505–10.
35. Frigolet ME, Torres N, Uribe-Figueroa L, Rangel C, Jimenez-Sanchez G, Tovar AR. White adipose tissue genome wide-expression profiling and adipocyte metabolic functions after soy protein consumption in rats. *J Nutr Biochem.* 2011;22:118–29.
36. Takahashi Y, Ide T. Effects of soy protein and isoflavone on hepatic fatty acid synthesis and oxidation and mRNA expression of uncoupling proteins and peroxisome proliferator activated receptor in adipose tissues of rats. *J Nutr and Biochem.* 2008;19:682–5.
37. Torre-Villalvazo I, Tovar AR, Ramos-Barragan VE, Cerbon-Cervantes MA, Torres N. Soy protein ameliorates metabolic abnormalities in liver and adipose tissue of rats fed a high fat diet. *J Nutr.* 2008;138:462–70.
38. Garait B. Le stress oxydant induit par voie métabolique (régimes alimentaires) ou par voie gazeuse (hyperoxie) et effet de la GliSODin®, Doctorat thesis in University of JOSEPH FOURIER. 2006. p. 196.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at
www.biomedcentral.com/submit

